

Exhibit B

Goal: Generate constructs to express the catalytic domains of murine Fuc-TIV and the sequence from phage 104 in bacteria. Protein generated in bacteria will subsequently be used to generate antibodies.

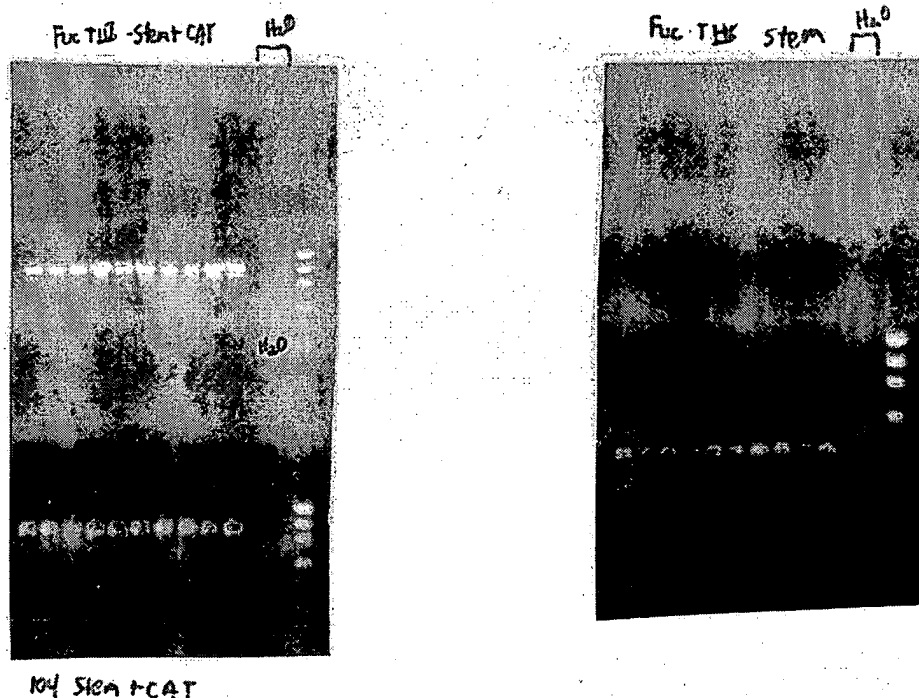
Experiment: Three constructs will be made by PCR: 1) Fuc-TIV stem+cat (primers 8966 and 623 B); 2) Fuc-TIV stem (primers 8965 and 623B); 3) 104 stem+cat (primers 624B and 625B). 104Sac-pTZ19 and pCDNA1-Nco/Ssp (50 ng) were used as template for each reaction

Amplification Scheme:

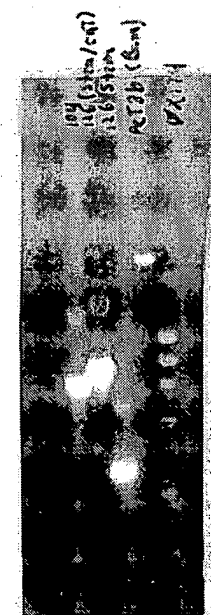
94 C	3.5 min	
94 C	1.5 min*	
72 C	2.5 min*	*20 cycles
72 C	8.0 min	
04 C	hold	

Each primer has BamHI sites at its 5' end. Using these sites, the inserts will first be subcloned into pet3b. After sequencing and confirming the absence of PCR mutations, the inserts will also be subcloned into the trpE path10 vector.

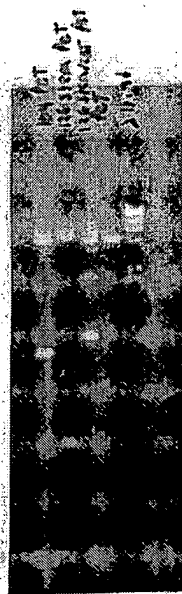
3 ul of each PCR reaction was analyzed on a 1% agarose gel. Samples were subsequently pooled, and butanol extracted, and ethanol precipitated. The samples and 10 ug of pet3b were subsequently digested with BamHI (50 ul reaction volume).



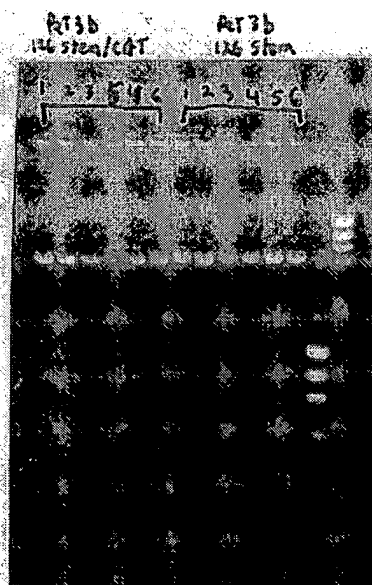
Following Bam digestion, pet3b was treated with 0.5 ul cip and incubated for 30 minutes at 37 C. PCR products were electrophoresed and purified from a 1% seaplaque gel by melting and phenol extraction. Inserts were resuspended in 11 ul of water and the vector was resuspended in 50 ul of water. One ul of each were run on a 1% gel for concentration assesment. Following analysis, 10 ul of water was added to each insert (20 ul total following gel). Ligations were set up using 1 ul of insert and 5 ul of the pet3b vector. A vector only control was included. Reaction were incubated at 15 C overnight. One ul of each ligation was analyzed on a 1% gel. One ul of each ligation was transformed into DH5 α by electroporation. One, 10 and 100 ul of each transformation was plated on LB amp plates (200 ug/ml). On the 100 ul plate, each insert transformation had approximately 100 fold more colonies (vector control had only 2). Six clones for each insert were selected for inoculation into LB and for minipreping. Miniprep DNA was resuspended in 20 ul water. Two ul were digested with BamHI in 20 ul total volume. Digested DNA was electrophoresed on a 1% agarose gel. All six stem clones had the insert, while only the 104 clones 2, 3, 4 and the cat clone 4 had inserts.



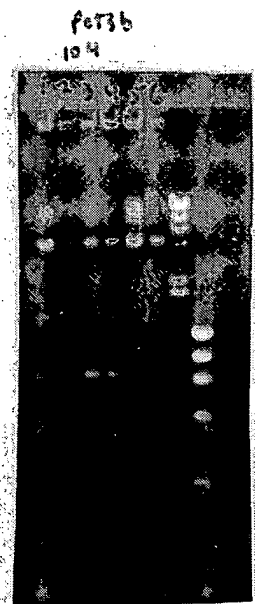
Gel purified vector and inserts



Post ligation



Bam digest of minipreps



Bam digest of minipreps